Biomarkers: Approachs/Status/ State of the Science

Jane Gallagher, Ph.D.

Environmental Health Scientist

Human Studies Division

NHEERL

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Biomarkers/Application to Risk Assessment

I. Introduction Biomarker Principles
II. Commonly Applied Exposure and Effects endpoints
III. Use of Human Surrogate Cells/fluids and markers
IV. Potential Application of CRA/biomarker using EPA's RSEI

Biomarkers/Application to Risk Assessment

V. Emerging Technologies and How they impact Development of Biomarkers Re: CRA

VI. Research needs/Discussion points for breakout

I. Introduction Biomarker Principles

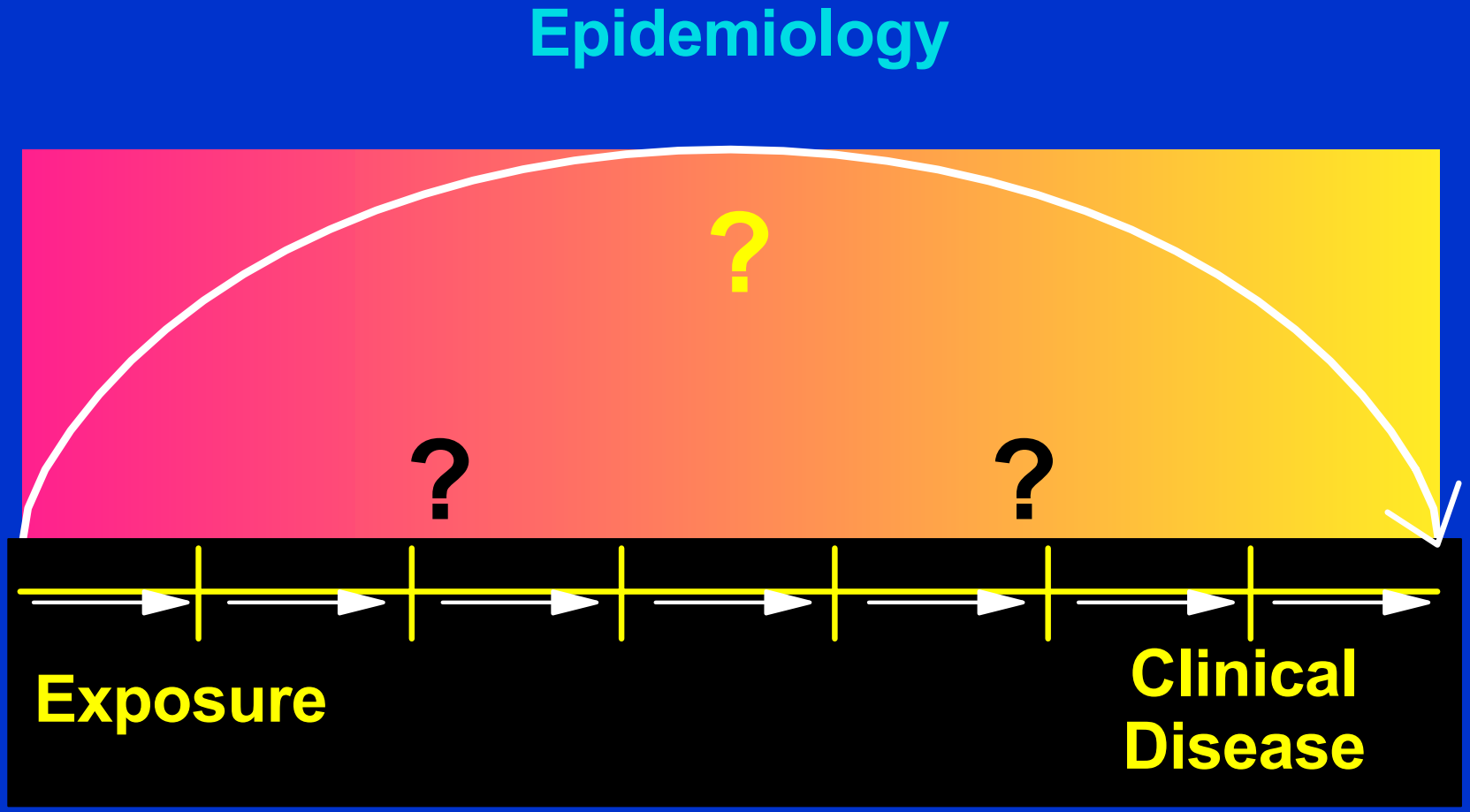
Biomarker

- An exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism NRC

Biomarker classification

- **Exposure**
  lead in the blood or urine
- **Effects**
  biological response to an exposure-
DNA adducts cell mutation
  Induction of Gene expression
- **Susceptibility**
  innate or induced capabilities for response to exposure

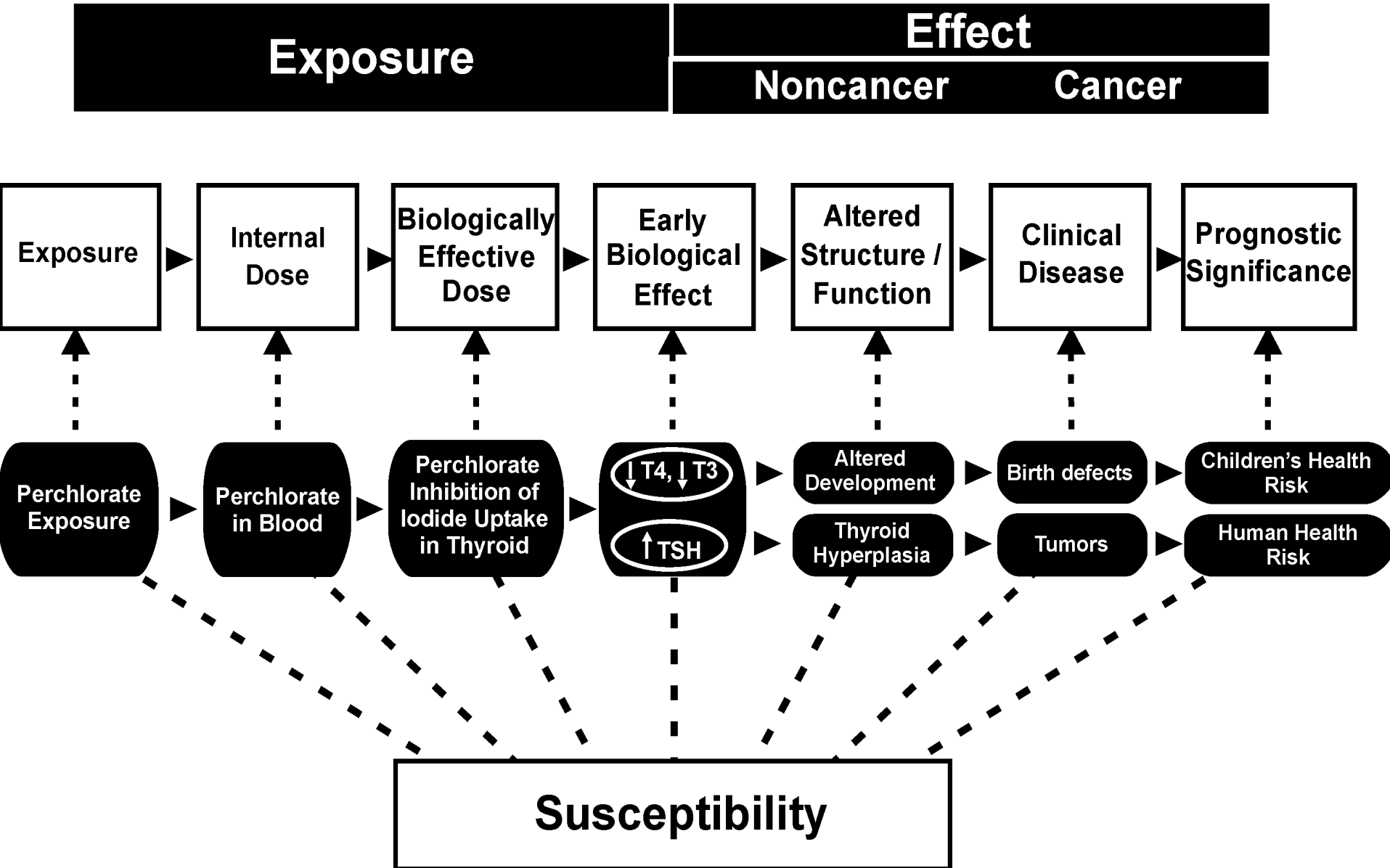
Where we are



- Ammonium perchlorate (AP) is the primary oxidizer in solid fuel used in rockets and missiles

- Contamination due to
  - Improper disposal
  - Incomplete OB/ODops
  - Firing of munitions
  - Fireworks
- Salts such as AP readily dissolve in water and anion (ClO_4^-)results
- Very stable, readily migrates through various media

Proposed Mode-of-Action Model for Health Risk Assessment of Perchlorate



Risk assessment: Importance of biomarkers

- Direct measurement can lower uncertainty in the RA process
- Provide insights into the possible shape of dose/response curve at levels below clinical observations
- Help identify subpopulations of individuals who may be at elevated risk

- Measures internal dose, reduces exposure misclassification and improves risk assessment

Factors affecting validation of biomarkers

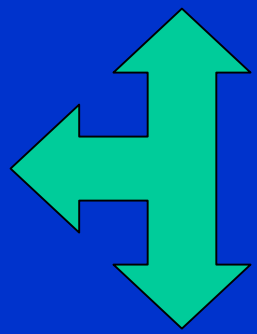
- Significance
- Specificity
- Sensitivity
- Knowledge of background in general population
- Knowledge of confounding factors
- Estimation of inter and individual variation

Factors affecting exposure responses

Activation

Detoxification

Elimination



susceptibility

Ethnic and racial groups – cancer incidence

- US African Americans have cancer incidence rates 3x esophageal cancer
- 2x liver, cervical and stomach and 50 % higher
- Caucasians have higher incidence for endometrial and postmenopausal breast cancer

Cancer Gene list impacting….

- DNA adducts
- DNA damage
- DNA replication
- Angiogenesis
- Behavior
- Cell cycle
- Cell signaling
- Metabolism
- Signal transduction etc…..

http://lpgws.nci.nih.gov/html-cgap/cgl/

Challenges / limitations

- Most biomarkers have a baseline response that is observed in population with no specific exposure to a toxic chemical
- Lack of validation of most biomarkers
- Method may not measure all sources
- Or may measure other exposures not of interest

Examples mode of action single chemicals

Formaldehyde → DNA cross links

d-Limonene → Alpha 2-uglobulin

Chloroform → cytotoxicity

More Examples

- BaP → DNA reactive metabolites
- Amitrole → Increased Thyroid
- Perchlorate → altered thyroid homeostasis
- Vinylacetate → cell proliferation

Examples for Classes of Chemicals

- PAH- DNA reactive intermediates
- Dioxins-- receptor mediated responses
- Estrogens-- receptor mediated responses
- Organophosphate----cholinesterase inhibition
- POM - DNA reactive intermediates – immune responses

Classes of compounds for which human DNA adducts have been observed

- PAH
- nitrosamines
- mycotoxins
- aromatic amines
- uv light
- alkylating cancer
- chemotherapeutic agent

Human Studies PAH as class of chemicals

- Populations in areas of Poland with highly polluted air compared to relative unpolluted rural area
- Associated with elevated DNA adducts
- SCE CA and RAS over expression…seasonal variation noted as well

Perera *et al*., 2001

Validation of Biomarkers in Remote Locations

II. Commonly Applied Exposure and Effects endpoints

Battery of endpoints capturing Net effect–over several mechanisms of action/classes

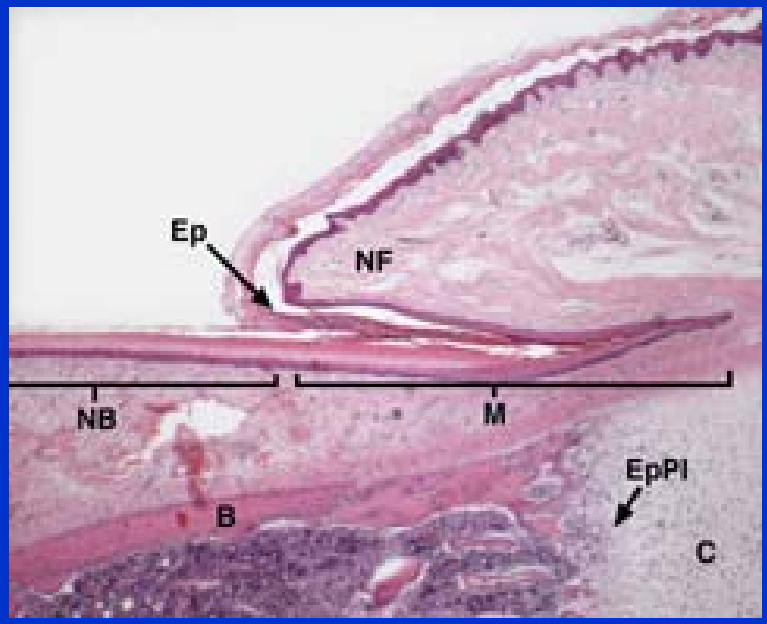
- Mutagenicity-urine
- Comet assay
- 1-OH pyrene
- Cholinesterase inhibition
- Cross-linking
- CA, SCE
- Glycophorin A
- HPRT
- DNA adducts **Mutagen sensitivity**
- **Oxidative damage**
- P53
- FISH

Human biological cells/fluids

- Nasal lavage fluid and cells
- Bronchoalveolar lavage fluid and cells
- Blood plasma and cells
- Cord blood plasma and cells
- Nails -DNA
- Semen
- Urine urothelial cells/bladder cells
- Milk epithelial cells and lipids
- Buccal Cells

III. Use of Human Surrogate Cells/fluids and markers

Infant nail



NF= Nail Fold
NB= Nail Bed
M= matrix of nail bed
Hy= hyponchium
E =Eponychium

MTHFR A1298C & MTHFR C677T

NAT2 G857A

GST-T1 & GST-P1

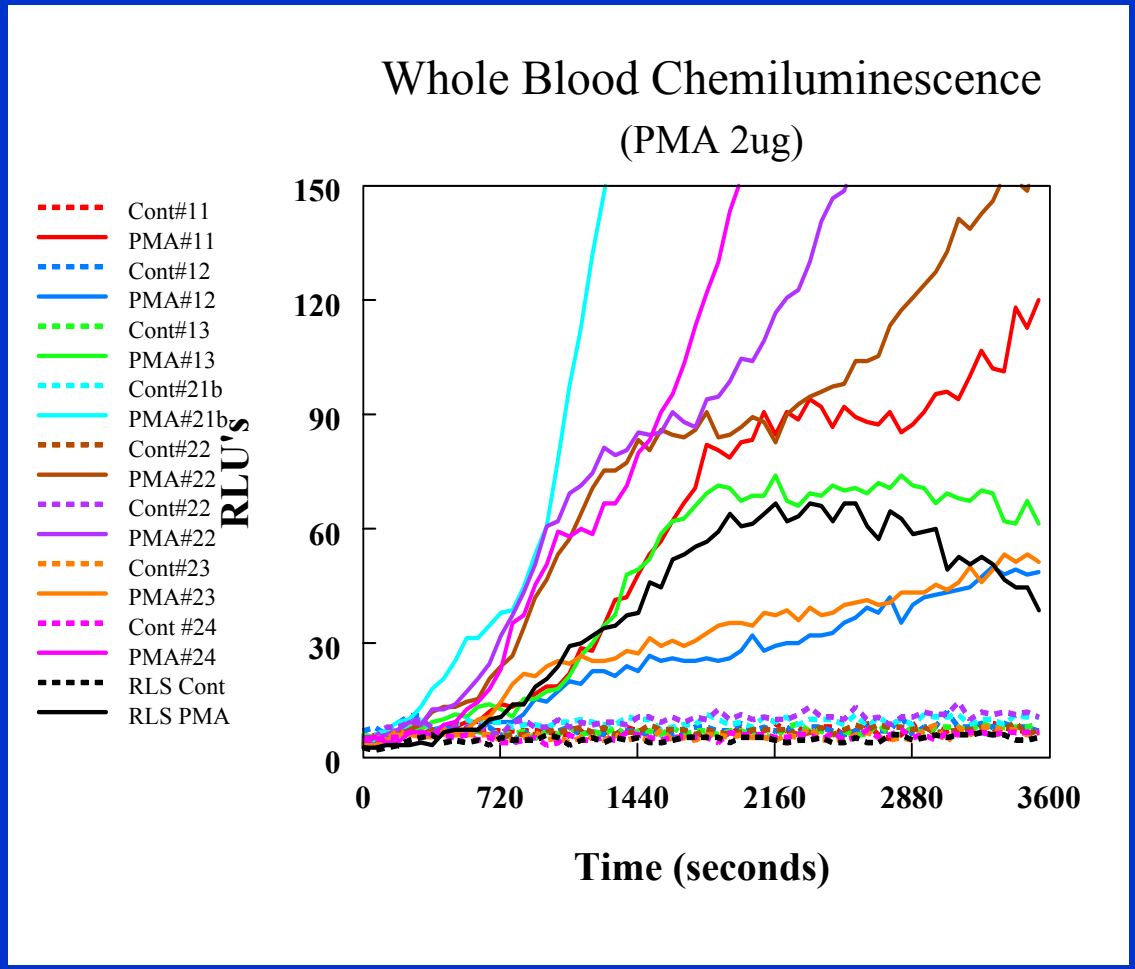
MPO G-463A

XPD

XRCC1

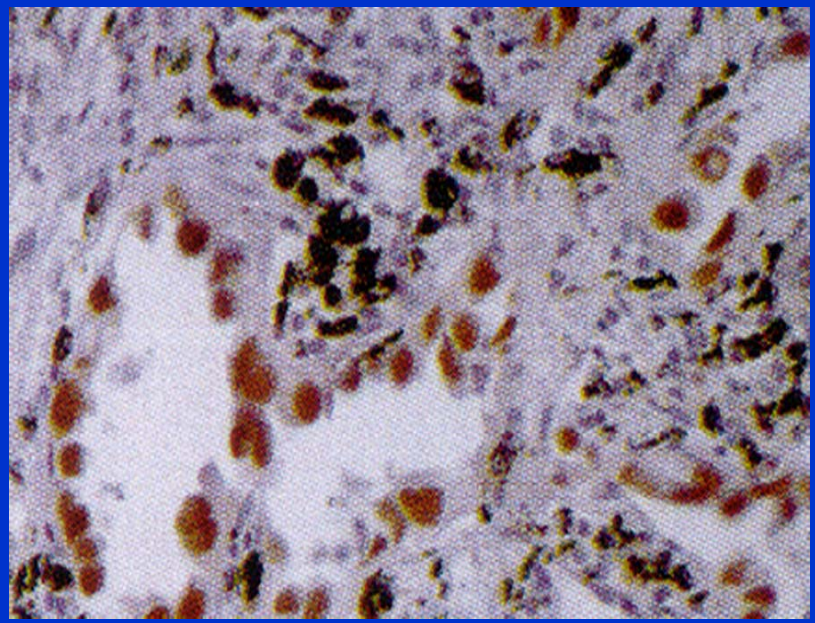
Oxidative Stress-Blood

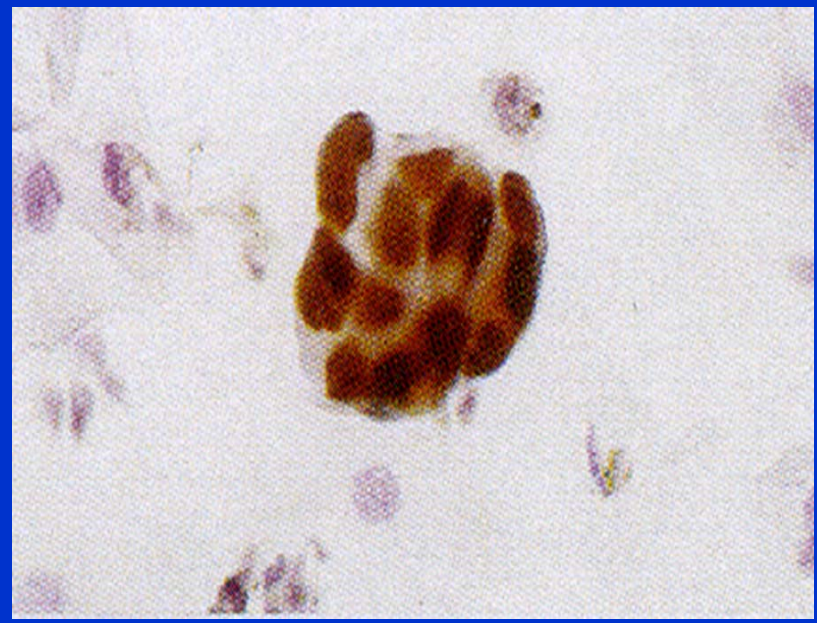
- PMA –phorbol myrastate acetate
- **Sams/Gallagher NCS pilot study**



Sputum

Detection of p53 protein accumulation in sputum and lung adeno carcinoma associated with indoor exposure to unvented coal smoke in china

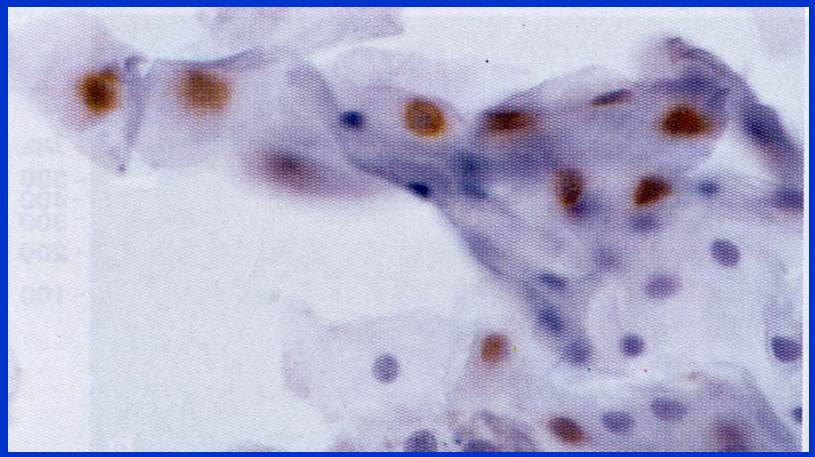


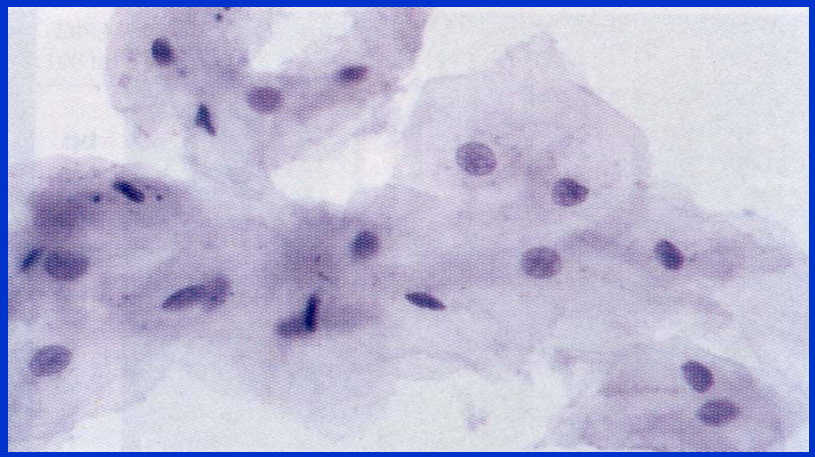


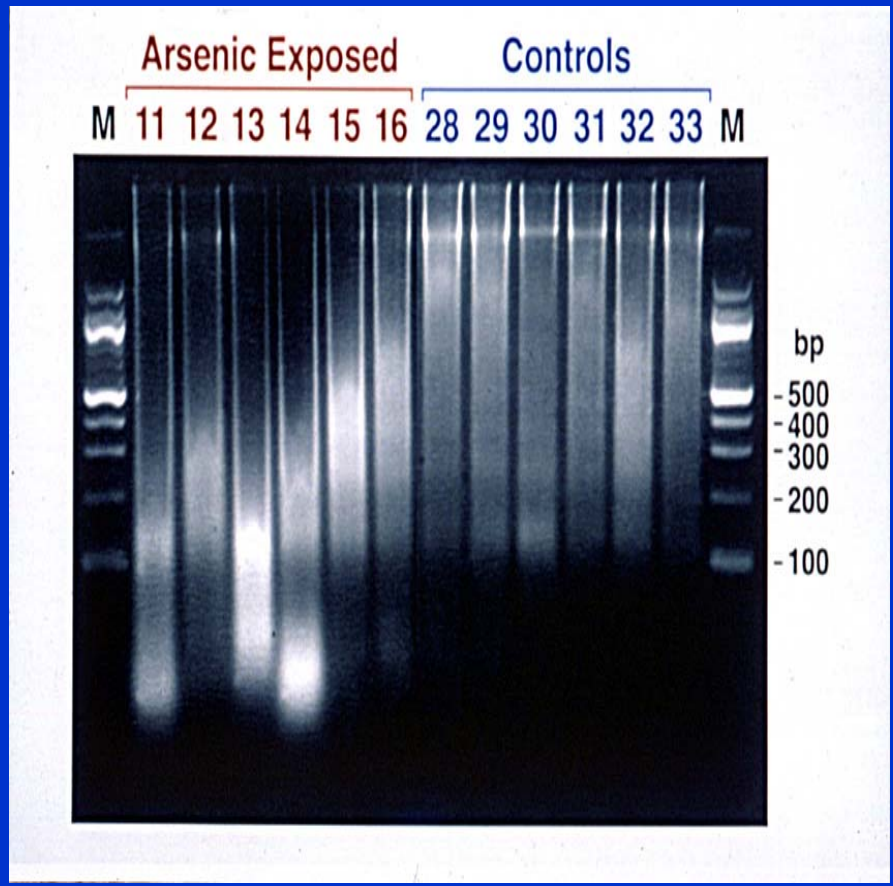
Feng et al.,19:3847-52, AntiCancer Research, 1999

Buccal Cells

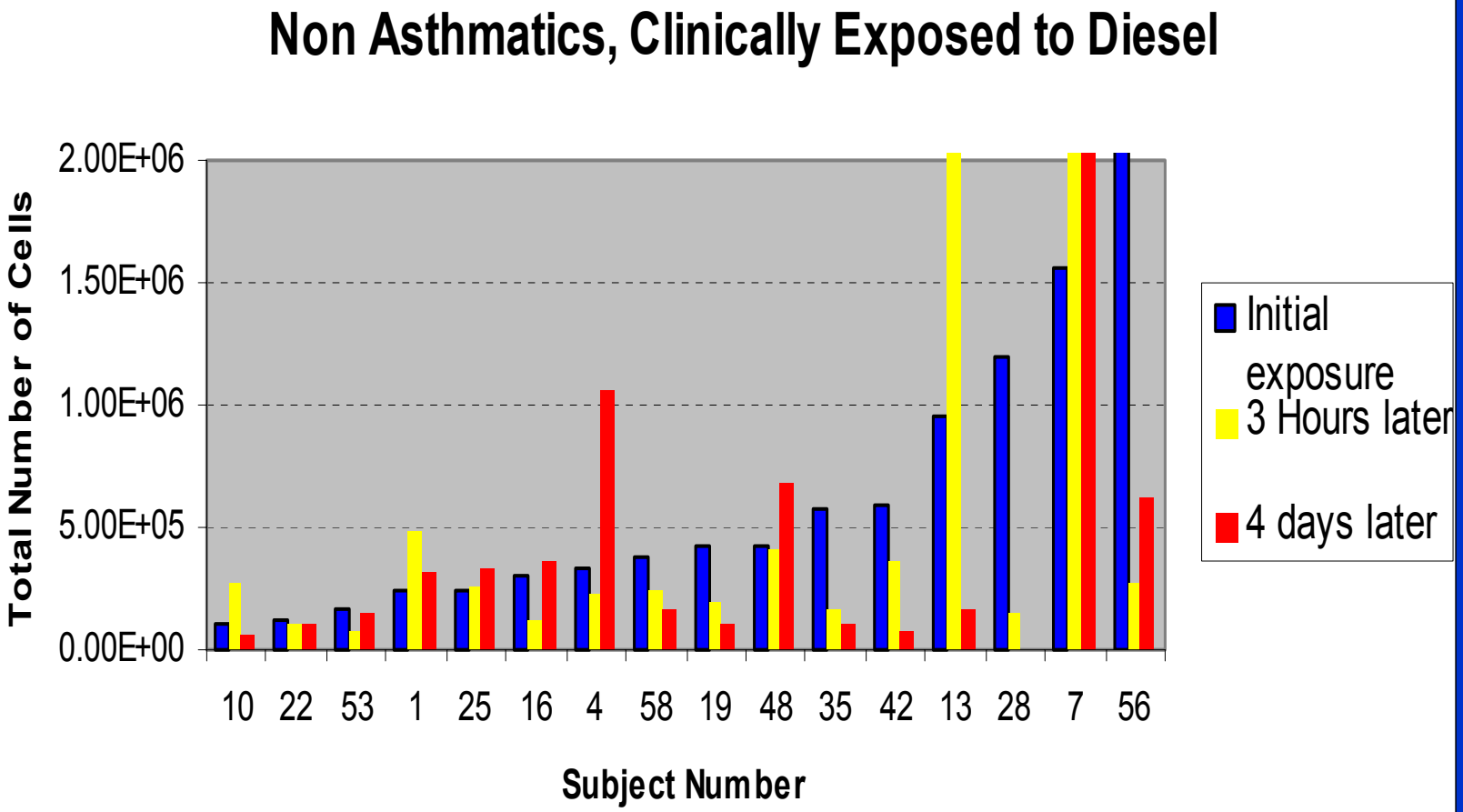
Buccal Cells: DNA Fragmentation by DNA Laddering Assay







Nasal Lavage



Breast Milk

Evidence for the Presence of Mutagenic Arlyamines in Human Breast Milk and DNA Adducts in Exfoliated Breast Ductal Epithelial Cells

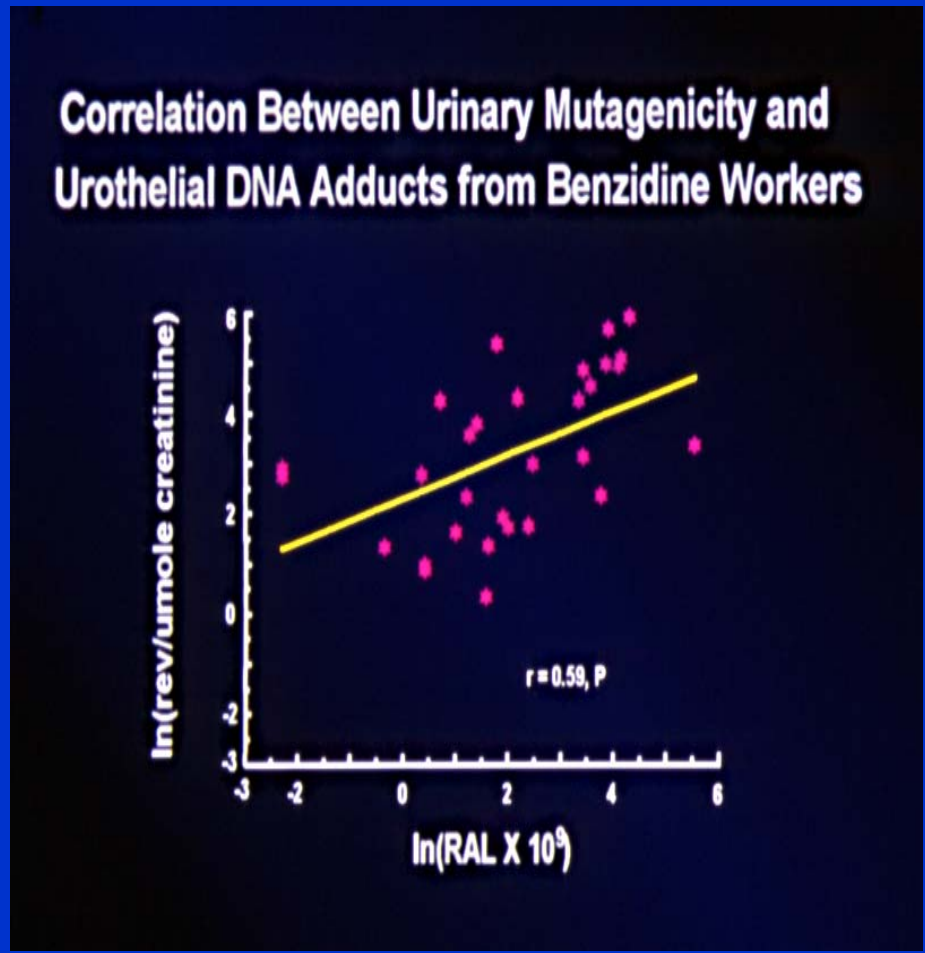
Thompson *et al*., Environ. Mol. Mutagen. 39:134-142, 2002

Results of Breast Milk Study

- 66% of exfoliated breast epithelial cells had DNA adducts using ^{32}P-postlabeling.
- 58% (14/19) of samples with adducts were also mutagenic, and 85% (11/13) of mutagenic samples had adducts.
- Bioassay-directed fractionation showed that most mutagenicity was in the basic fraction, suggestive of arlyamines, probably from the diet.
- 88% (22/25) breast milk samples were mutagenic using *Salmonella* YG1024.

Urine

- Correlation Between Urinary Mutagenicity, Urinary Metabolites, and Urolethial Cell DNA Adducts Among Benzidine-Exposed Workers



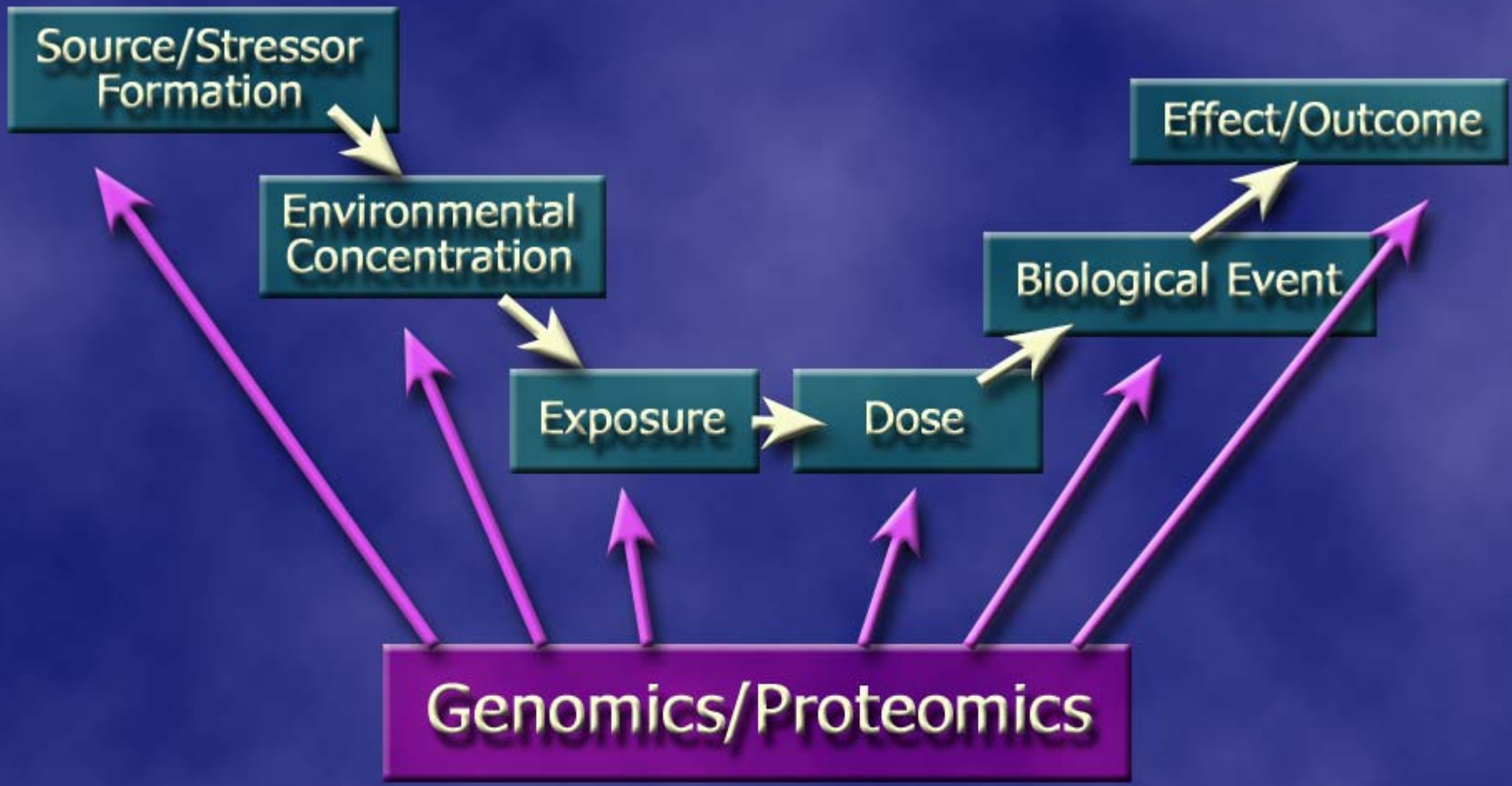
DeMarini *et al*., Carcinogenesis 18:981-988, 1997

V. Emerging technologies impact biomarker and CRA

- ICP-MS metabolites and adducts
- FISH fluorescence in situ hybridization from the chromosomal level to specific genes related to the disease process
- PET positron emission tomography measures change at a molecular scale
- **SNP** in genes-involved in disease
- High output technologies –**DNA micro array gene** function and expression
- Proteomic technologies

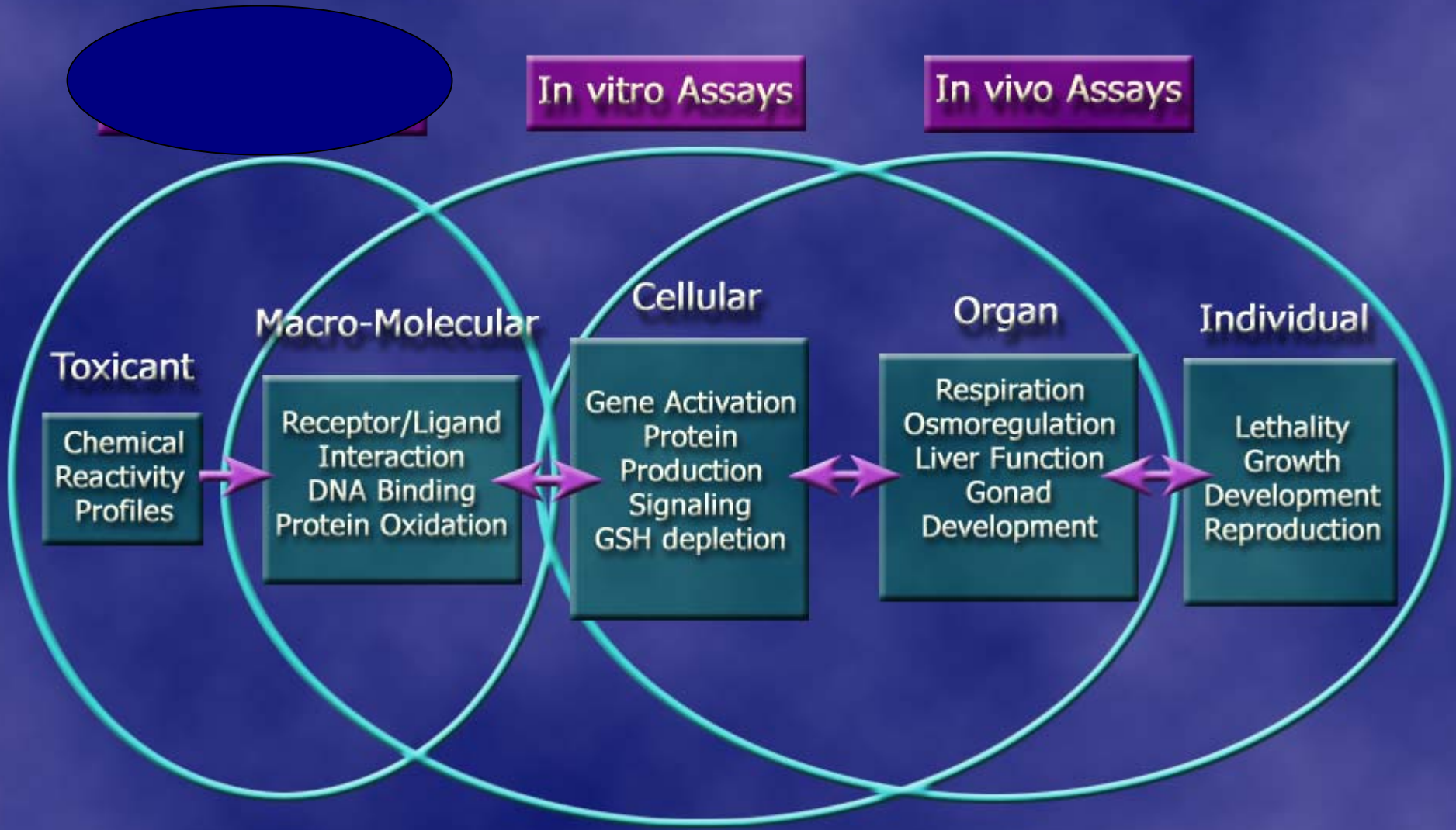
Definition of Genomics

"study of all the genes of a cell or tissue, at the DNA (genotype), mRNA (Transcriptome), or protein (proteome) levels and can aid in understanding normal, adaptive and abnormal cellular function"

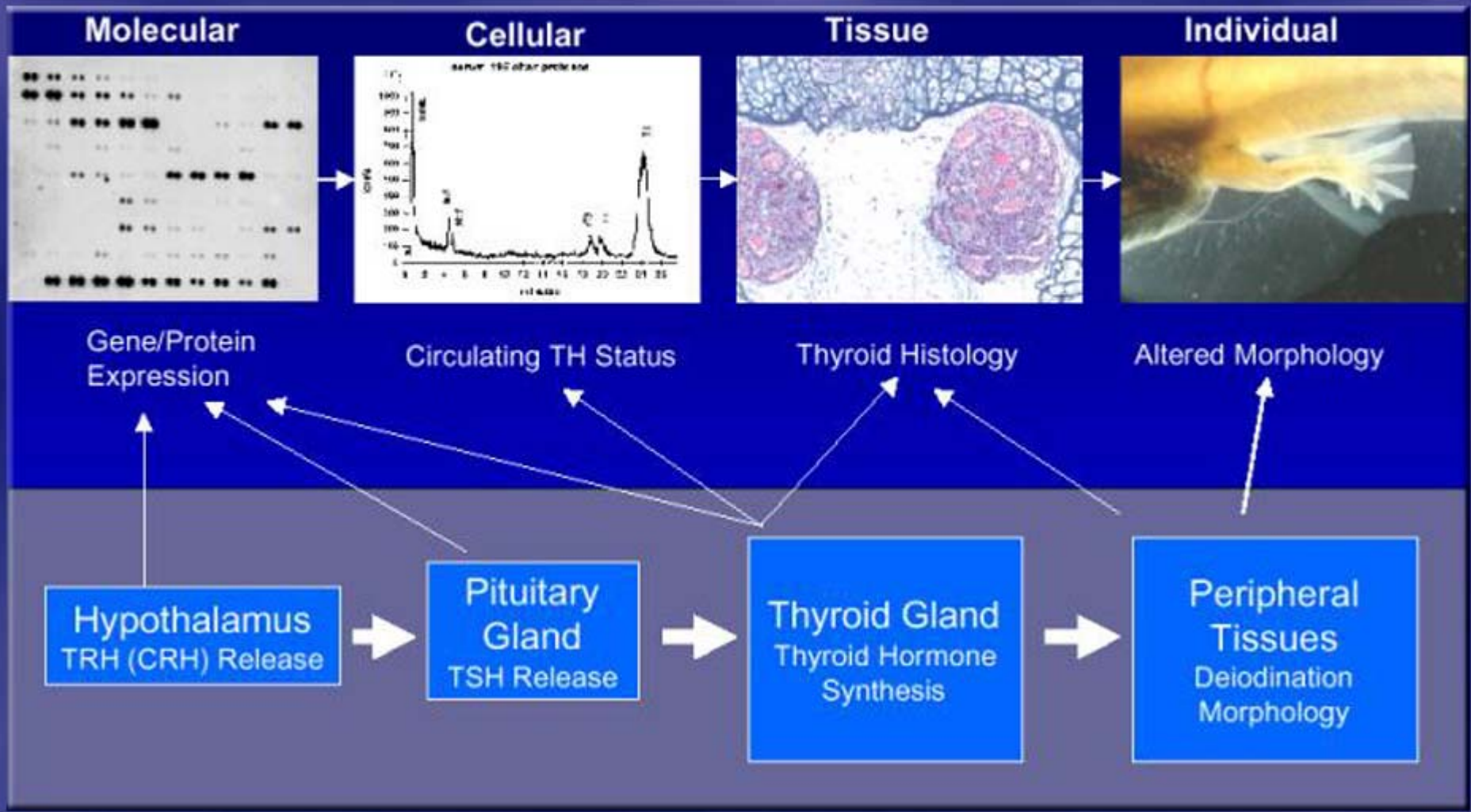

Source/Stressor Formation
Environmental Concentration
Exposure
Dose
Biological Event
Effect/Outcome
Genomics/Proteomics

Toxicity Pathways

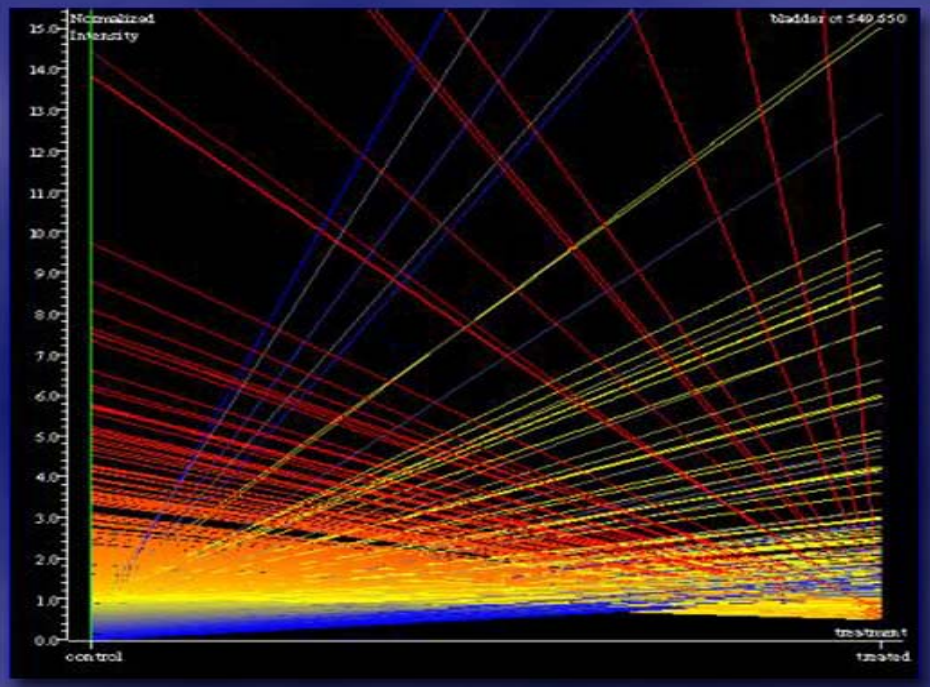
Linking Observations Across Levels of Biological Organization



Xenopus Metamorphosis Model for Thyroid System Disruption

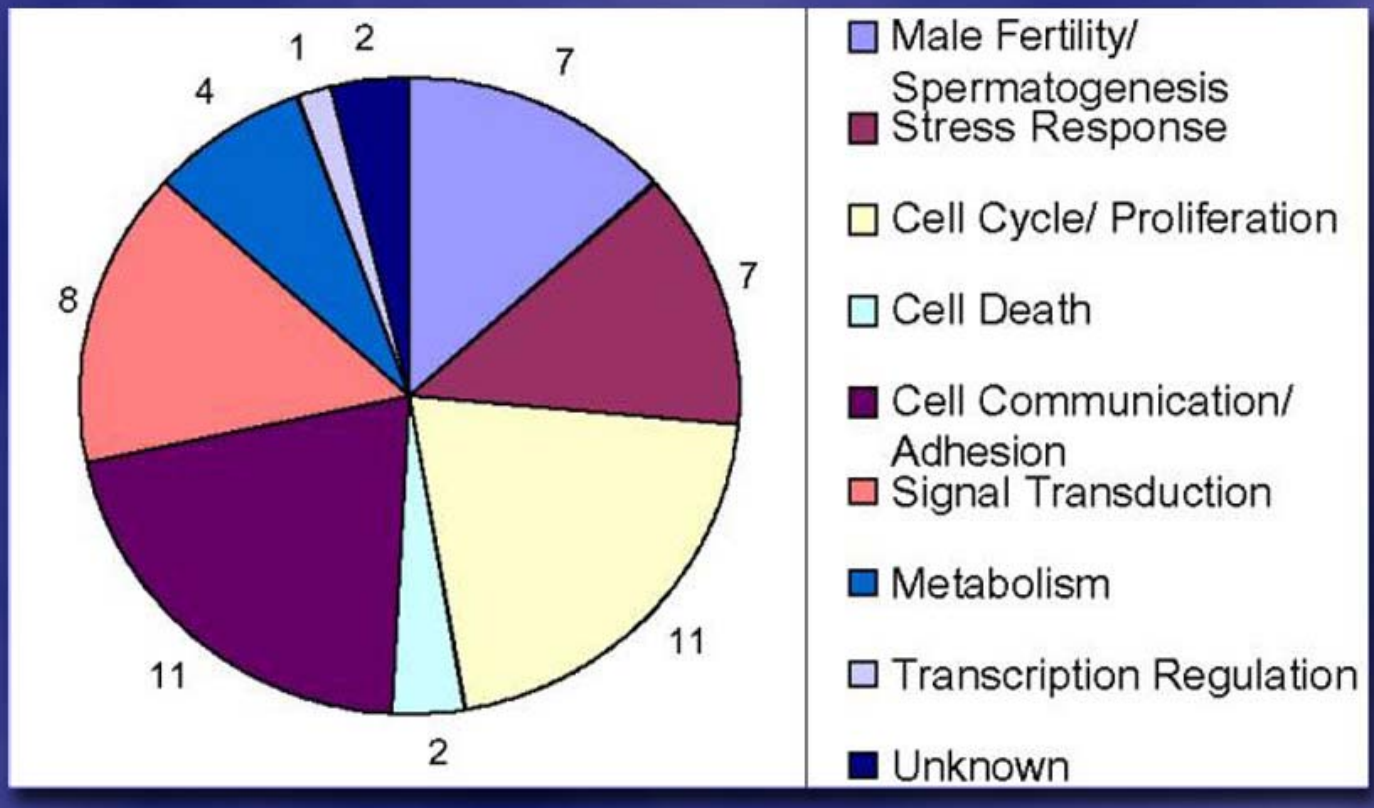


Gene Expression



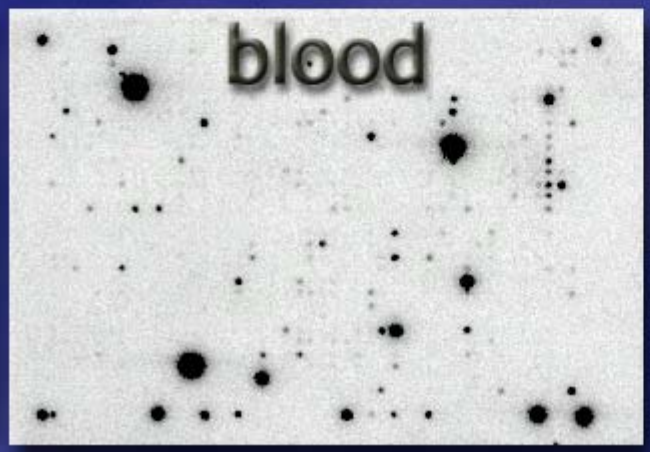
Transcriptional alterations in the transitional epithelium after 21 days of treatment with MX in the drinking water. 194 (16%) of the genes on the 1.2K array are increased 5-fold or more and 38 (3%) are decreased 5-fold or more.

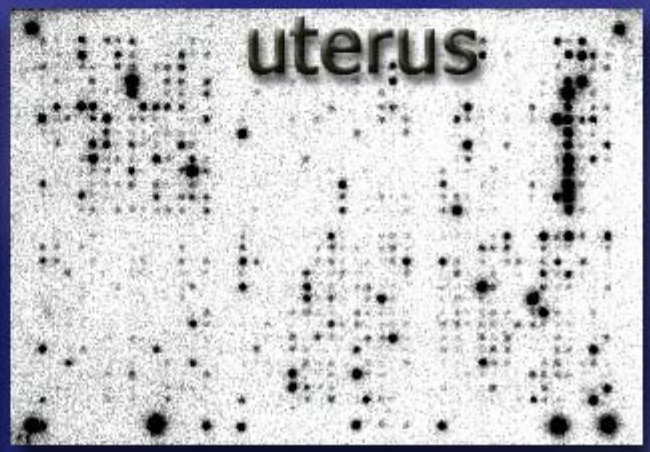
Toxicogenomics of Water DBPs: 53 Genes Affected in Mouse Testis Clustered by Biological Process



Gene Expression in Blood and Uterus of Rats Following Estradiol Exposure

- 193 genes detectable in both blood and uterus.
- 18 genes significantly altered in both blood and uterus.
- Agreement of gene expression changes with literature on estradiol effects.
- Gene expression changes consistent with uterotrophic effect.





"WHEN THE RUBBER HITS THE ROAD!"

- MORE **IMMEDIATE** NEEDS FOR EXPOSURE and CRA MODELS --
- Application re: **EPA Regional offices**

WHAT MODELS ARE USEFUL TO THE REGIONAL OFFICES (CRA) ???

1) FROM A CUMULATIVE RISK ASSESSMENT POINT OF VIEW ???

2) CAN WE USE THEM FOR BIOMARKER DEVELOPMENT/VALDIDATION ???

RSEI Risk Screening Environmental indicators

- OPPT
- Prioritization for strategic planning, risk related targeting and trends averaging
- **Tracks potential risk related impacts of chemical releases and transfers**
- NATIONAL/STATE/REGIONAL/ COUNTY
- Who currently is using this ??

RSEI-biomarkers
Models Air and surface water

- Addresses cancer and noncancer effects – independently or in combination
- Developmental toxicity, reproductive, toxicity neuro toxicity
- NOT Acute toxicity

RSEI Components

- Exposure assessment (air and surface water)
- **Distribution and # of people**
- Source apportionment
- Target dose
- Emission source
- Pollutant type amt location
- **Environmental concentration air and water**
- Environmental Justice

Indicator Element

- Indicator Element=
- Toxicity Wt x Surrogate Dose x Exposed Population
- Overall Indicator Value sums all relevant individual chemical- facility-medium specific indicator elements
- Provide magnitude of population not ind.risk

RSEI
Chronic Human Health Indicator

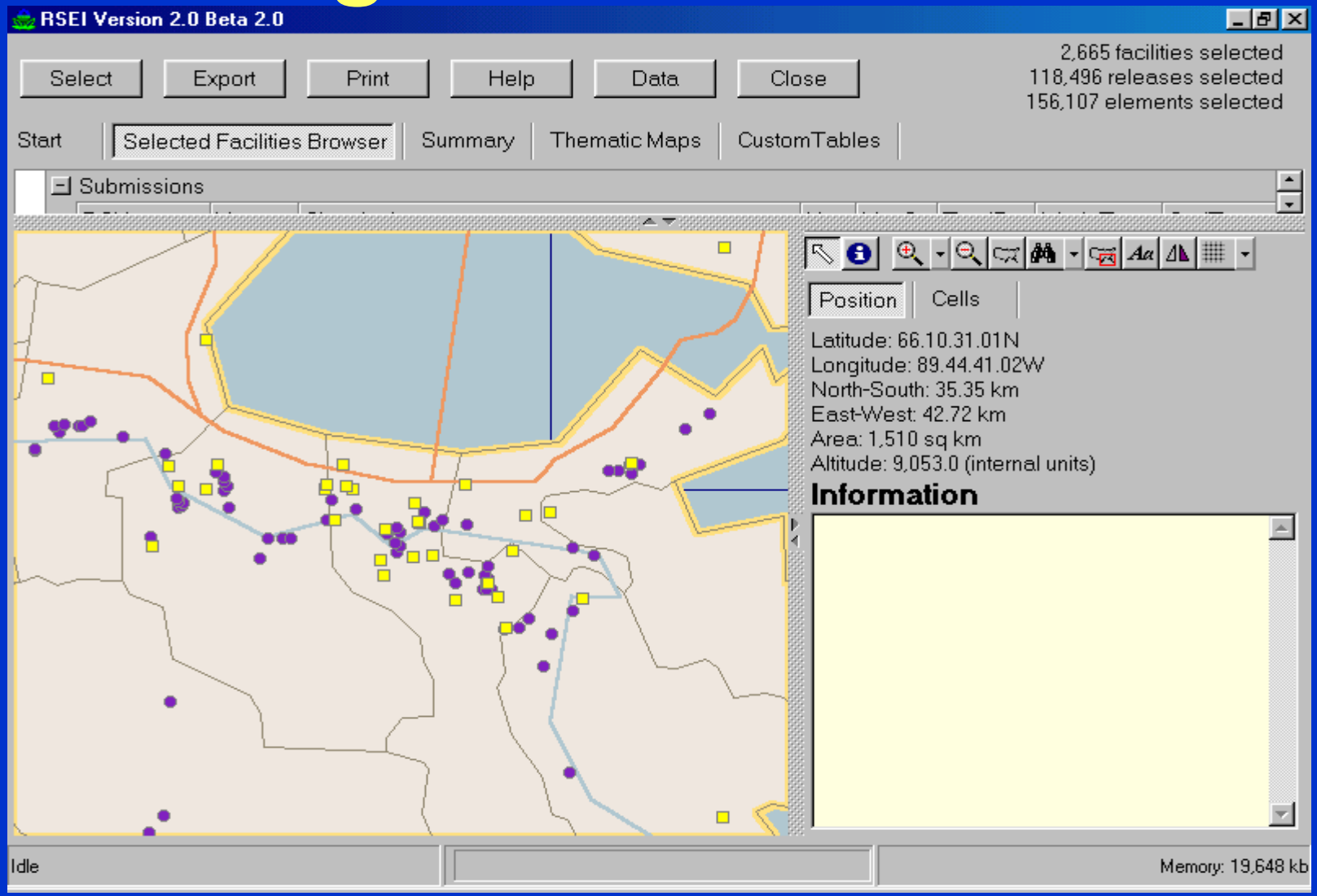
(order of preference)

- IRIS(EPA integrated risk information system
- EPA office Pesticides
- Programs toxicity tracking report
- EPA Health effects Assessment summary tables
- Final derived or interim derive toxicity weight estimated by OPPT

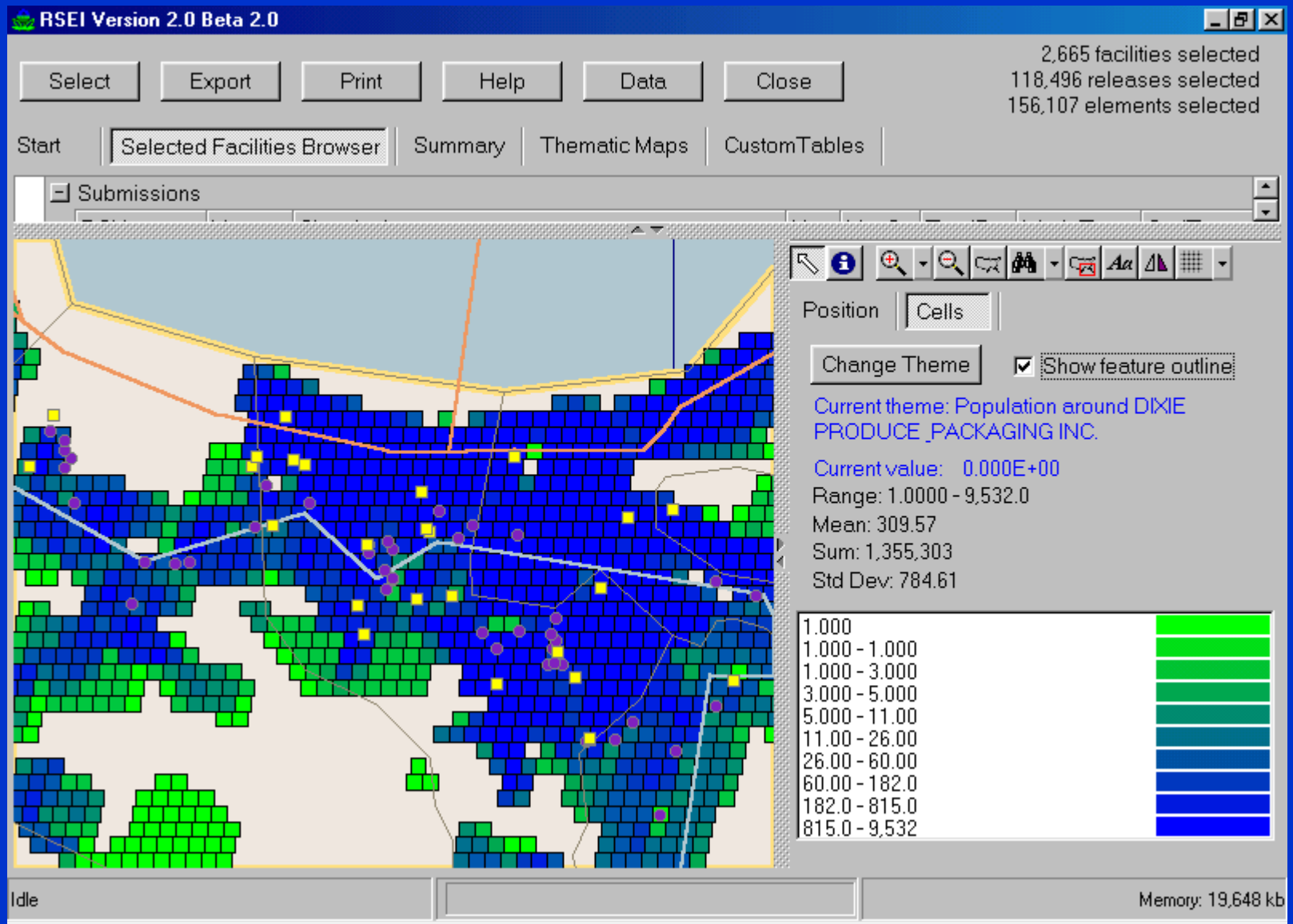
How related to biomarker application/validation ?

- OSHA CARCINOGENS
- METALS
- HIGH PRODUCTION VOLUME Chemicals
- Priority Chemical under BIO-ACCUMULATIVE and Toxic Chemical Program
- Chemical Specific

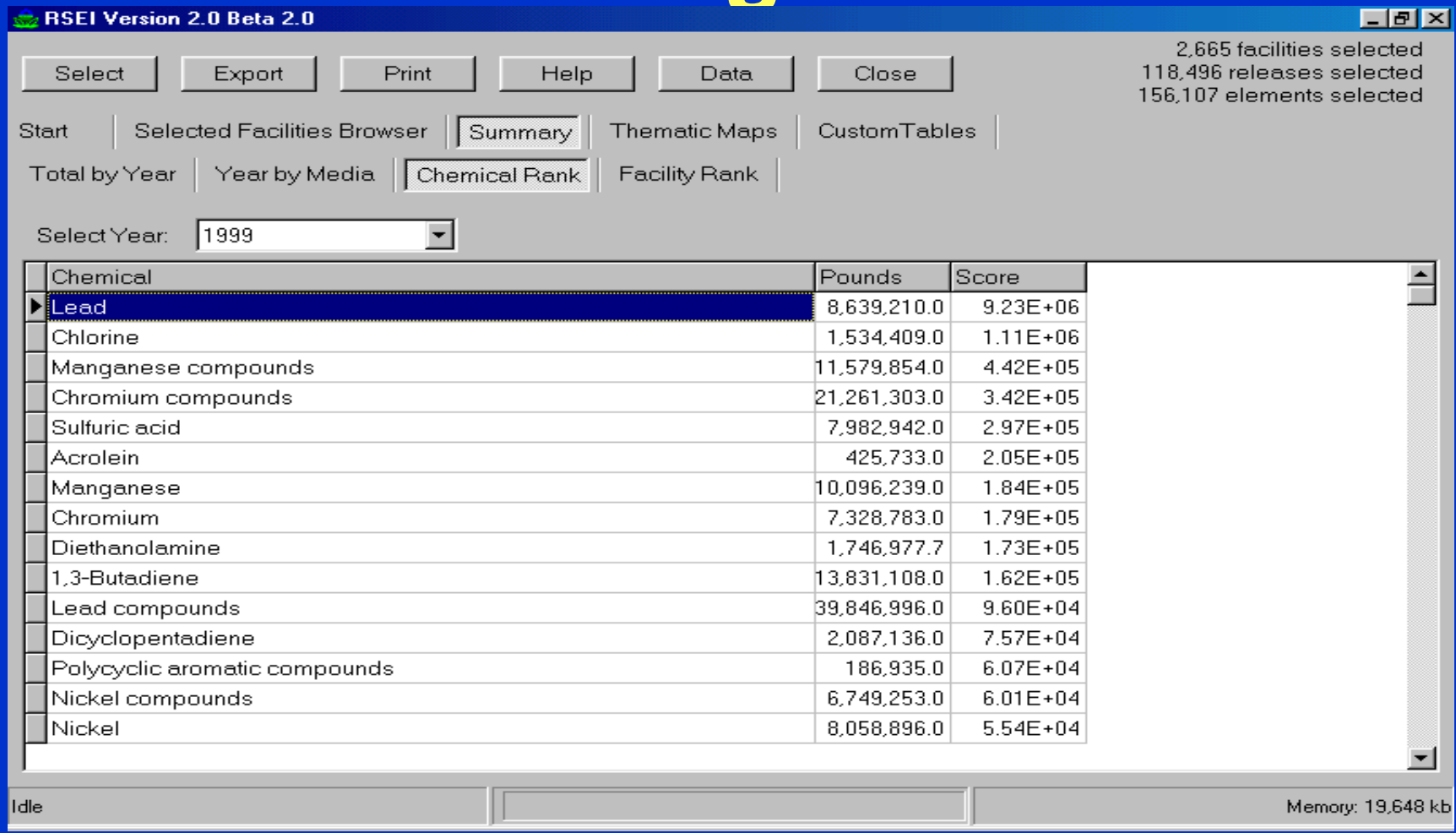
Distribution of Facilities, Region 6



Population Distribution, Region 6 (Elderly & Children)



Chemical Ranking by Indicator Score and Lbs, Region 6



Chemical	Pounds	Score
Lead	8,639,210.0	9.23E+06
Chlorine	1,534,409.0	1.11E+06
Manganese compounds	11,579,854.0	4.42E+05
Chromium compounds	21,261,303.0	3.42E+05
Sulfuric acid	7,982,942.0	2.97E+05
Acrolein	425,733.0	2.05E+05
Manganese	10,096,239.0	1.84E+05
Chromium	7,328,783.0	1.79E+05
Diethanolamine	1,746,977.7	1.73E+05
1,3-Butadiene	13,831,108.0	1.62E+05
Lead compounds	39,846,996.0	9.60E+04
Dicyclopentadiene	2,087,136.0	7.57E+04
Polycyclic aromatic compounds	186,935.0	6.07E+04
Nickel compounds	6,749,253.0	6.01E+04
Nickel	8,058,896.0	5.54E+04

Ranking Industries by Indicator Score, Region 6

RSEI Version 2.0 Beta 2.0

Select | Export | Print | Help | Data | Close

2,665 facilities selected
118,496 releases selected
156,107 elements selected

Start | Selected Facilities Browser | Summary | Thematic Maps | CustomTables

Total by Year | Year by Media | Chemical Rank | Facility Rank

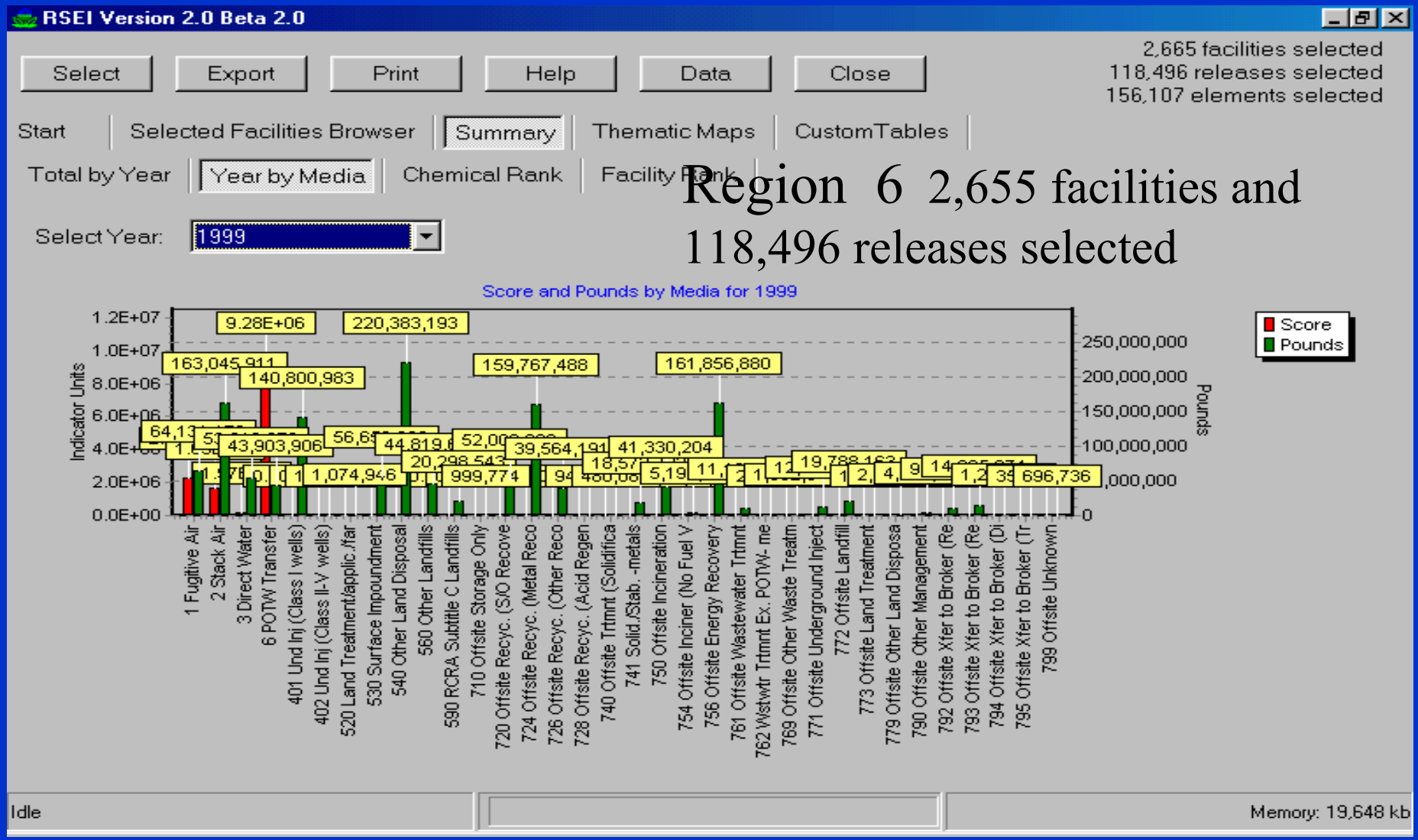
Select Year: 1999

ID	Name	Pounds	Score
75461CMPBL500N	CAMPBELL SOUP SUPPLY CO.	172,005.0	9.21E+06
70183DXPRDGST	DIXIE PRODUCE & PACKAGING INC	326,026.0	6.20E+05
79922MRCNM3666	AMERICAN MINERALS INC.	53,179.0	5.85E+05
75941DBLLF600AS	DIBOLL FIBER PRODS.	497,693.0	1.28E+05
77651TXCCHHWY	HUNTSMAN CORP. - C4/O&O PLAN	2,226,267.0	1.18E+05
77506CRWNC111F	CROWN CENTRAL PETROLEUM C	712,191.9	9.79E+04
71135FRYMS8700L	FRYMASTER L.L.C.	4,607.0	8.64E+04
71730LNLRF1000M	LION OIL CO.	1,082,869.0	6.76E+04
70805XXNBT4050S	EXXONMOBIL REFINING & SUPPLY	5,775,700.0	6.75E+04
77541THDWCBUIL	DOW CHEMICAL CO. FREEPORT	9,055,150.0	5.39E+04
70805XXNCH4999S	EXXON CHEMICAL BATON ROUGE	5,117,910.0	5.36E+04
77536SHLLLHIGHV	SHELL CHEMICAL L.P.	2,680,150.0	5.32E+04
78403CSTLR1300C	COASTAL REFINING & MARKETIN	905,394.0	5.30E+04
70079MTVNR1553I	MOTIVA NORCO REFINING CO.	1,034,040.0	4.92E+04
70068DPNTD560H	DUPONT DOW ELASTOMERS L.L.C	557,815.0	4.87E+04

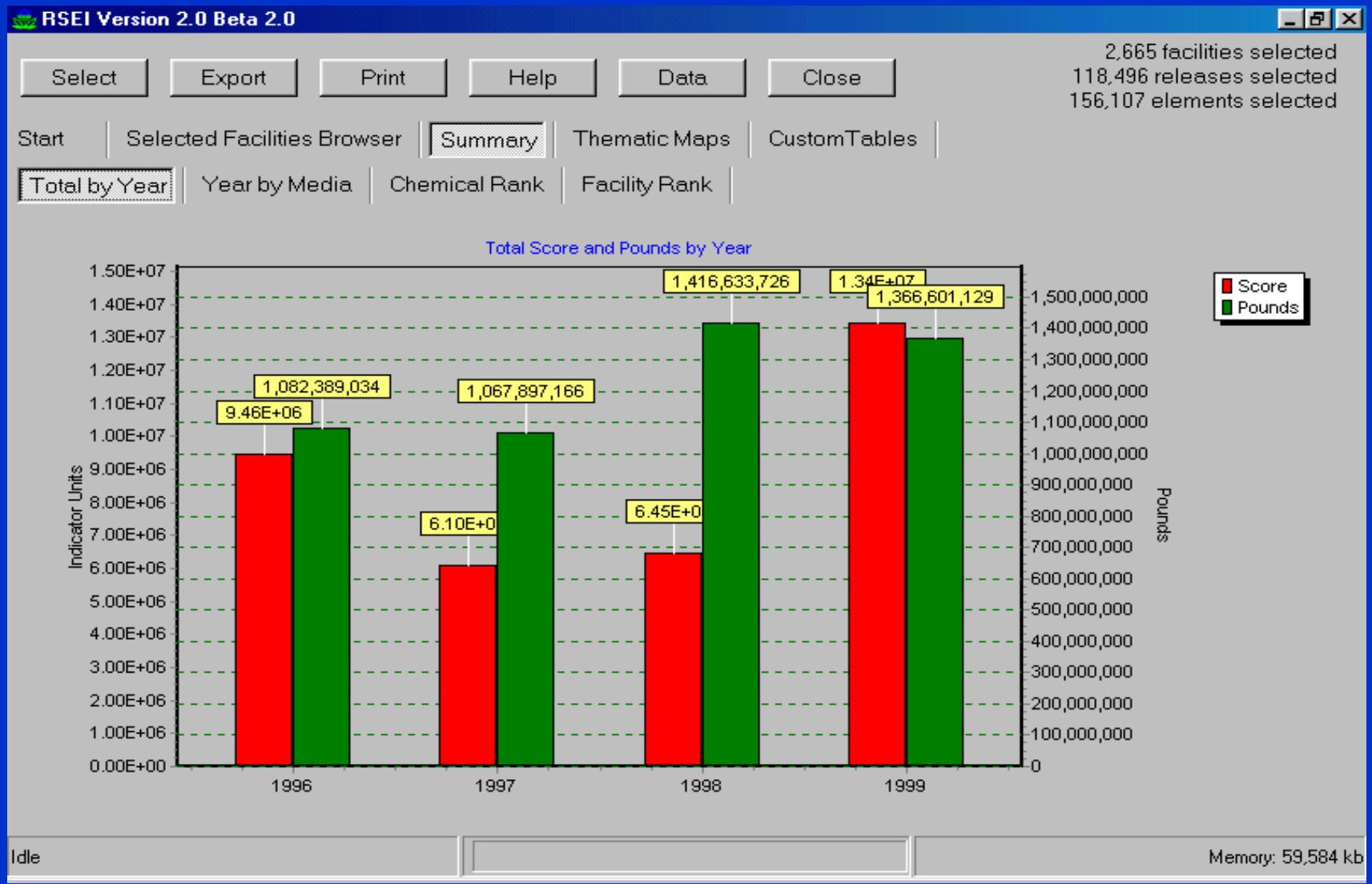
Idle

Memory: 19,648 kb

Indicator Score in Lbs and Media, Region 6



Trends by Indicator Score and Lbs EPA REGION 6



Trends by indicator score and Lbs EPA REGION 4

Clinical/Environmental Studies with biomarker/gene/environment components

- Nordic Collaborative Study
- Albertini –prospective study
- NHEXAS
- PEW Environmental Commission Study
- NCS
- Maryland case control Study
- BIOMAR International

PEW Environmental Health Commission

- Funded by a grant to the JH school of public health from Pew Charitable Trust
- Nationwide baseline tracking of
- Monitoring of immediate health crisis such as heavy metal and pesticide poisoning
- Develop a dedicated state and local rapid response capability to investigate district outbreaks and emerging treads

Biomarker Needs

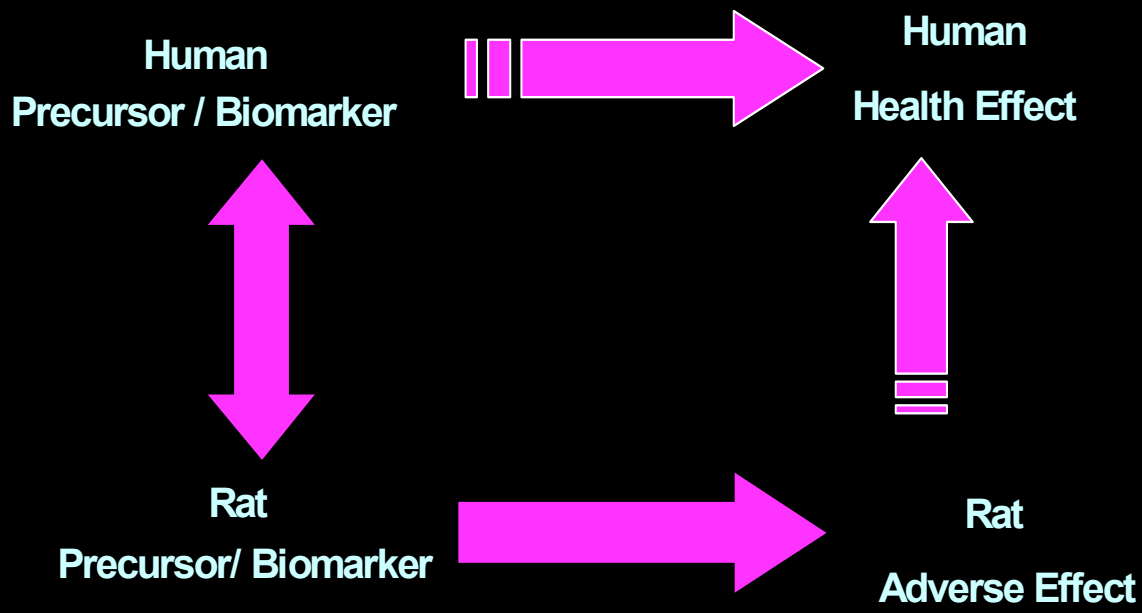
- Exposure biomarkers needed that identify a greater variety of chemical and metabolites
- Battery of assay and endpoint covering the continuum
- Mechanistic information needed
- Archiving of biological and environmental samples and measurements as new technologies advance
- Validation of surrogate cells with target tissue responses

DISCUSSION POINTS
Biomarker

Risk Characterization *in vitro* Mutagenicity Sensitivity Assay

- Phenotypic characterization of sensitivity
- **Bleomycin**….strand breaks
- DNA repair characterization-BPDE
- Associations with lung cancer, head and neck and breast cancer

Use of Biomarker Data in Parallelogram Extrapolation to Human Homology



Jarabek (In preparation)

Comet assay

- Complimentary to mutagen sensitivity assays
- Phenotypic repair test
- Double strand breaks and single strand breaks
- Lung cancer patients reduced repair capacity compared to control.

NHGR, CDC, NIEHS

DNA Polymorphism Discovery resource of samples from 450 residents from major regions of the world.

Valuable for the discovery of human genetic variation with follow up studies can be performed related to health and disease.

SNPs

The National Human Genome Research Institute

Creating resource for the identification of SNP maps

Subjects can be typed for up to 100,000 SNP markers in the search for disease associated genes.

RSEI-toxicity wt

- A wt proportional number applied to a chemicals bases on its toxicity
- Chemical wt bases on their single most sensitive adverse health effect
- Range of toxicity wts .1 –1,000,000 for carcinogens
- .001-100,000 for non cancer

Smokey Coal

Lung tumor KRAS and TP53 Mutations in nonsmokers reflect exposure to PAH Rich coal combustion emissions

- DeMarini *et al*., Cancer research, 61:6679-81,2001

p53 mutational load Serum and tissue specimens

- Mutations in p53 tumor suppressor gene common in human cancer
- Measuring p53 in blood may indicate previous carcinogen exposure and identify individuals at increase cancer risk